

# Latex Allergy within a Cohort of Not-at-Risk Subjects with Respiratory Symptoms: Prevalence of Latex Sensitization and Assessment of Diagnostic Tools

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## Key Words

Asthma • Hev b allergens • Latex allergy • Rhinitis • Urticaria

## Abstract

**Background:** Immunoglobulin E (IgE)-mediated hypersensitivity to natural rubber latex (NRL) is a major problem in allergy practice. Currently, the use of skin prick tests (SPTs) with latex extracts and specific IgE detection for the diagnosis of NRL allergy in suspected patients is directed to identification of risk factors. Many cases of NRL allergy remain undiagnosed due to misreporting of symptoms by the patients or lack of proper questions asked by the physician. **Materials and Methods:** A total of 6,126 subjects referred for respiratory symptoms underwent SPTs with NRL. Positive subjects were resurveyed for exposure to NRL, and specific IgE for NRL extracts and recombinant molecules was determined. Immunoblots of NRL extracts were performed to identify IgE patterns. **Results:** Forty-six of 3,930 sensitized subjects had a positive SPT with NRL, displaying a prevalence of NRL sensitization of 0.75% for the general and 1.2% for the sensitized population. Eleven out of 46 (23.9%) subjects could be defined as NRL asymptomatic, whereas 35 (76.1%) developed symptoms upon exposure to NRL. Specific IgE to NRL was detected for 22 (75.86%) of 29 tested sera. Seventeen out of 22 (77%) sera displayed specific IgE to recombinant allergens

with most reactions to Hev b 5, Hev b 6.01 and Hev b 6.02. Immunoblots of NRL extract fractions with patients' sera showed heterogeneous patterns. **Conclusions:** SPTs with NRL extract should be routinely performed in patients with respiratory symptoms. Hev b 5, Hev b 6.01 and Hev b 6.02 are the most important allergens, but further characterization of NRL extracts is needed to identify novel allergens and to clarify the role of crossreactive carbohydrate determinants.

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## Introduction

Immunoglobulin E (IgE)-mediated hypersensitivity to natural rubber latex (NRL) is a major clinical problem in allergy and is often encountered in pediatric subjects undergoing repeated surgical procedures, such as those affected by spina bifida [1] and urogenital abnormalities [2], as well as in health care workers (HCW) and rubber industry workers as an occupational condition [3]. Sensitization may occur by wound exposure, by skin or mucosal contact, or inhalation of allergens from NRL-containing products. Elicited symptoms may encompass mucosal swelling, local or generalized urticaria, respiratory symptoms such as rhinoconjunctivitis and bronchial asthma, and anaphylactic shock [3].

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1018–2438/07/1432–0135\$23.50/0

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The epidemic of NRL allergy peaked in the 1980s and 1990s, most probably as a direct consequence of universal precautions implemented to contain the spread of HIV and other major blood-borne pathogens, through the use of NRL gloves [4]. The prevalence of sensitization to NRL is about 2–17% among exposed HCW, up to 30–50% in childhood patients with spina bifida [1, 5], but less than 1% in the general childhood [6] and adult [7] population. The prevalence of NRL allergy is actually lower, because some individuals sensitized to NRL, as assessed by in vivo and in vitro testing, do not have evident clinical symptoms [8].

Low-powder, powder-free and non-NRL gloves have been proven to be effective measures to minimize occupational NRL exposure and sensitization [9]. The incidence of NRL allergy in HCW in industrialized countries in Western Europe and North America is now decreasing [10, 11], but there are raising concerns that it will increase quickly in the near future in developing countries with fast-growing economies [12].

NRL is not only contained in a wide variety of medical devices, but also in several products and articles used in everyday life activities. Therefore, hidden sources of NRL in the working environment or in the household [13, 14] can virtually sensitize individuals in the general population by contact or inhalation, regardless of occupational exposure [15].

So far, 13 different NRL allergens have been cloned and sequenced and are referred to as Hev b allergens [16, 17]. Hev b 1 (rubber elongation factor) and Hev b 3 were described as major allergens for children with multiple congenital anomalies [18]. Hev b 2 ( $\beta$ -1,3-glucanase), Hev b 4 and Hev b 13 are important sensitizing allergens for HCW with NRL allergy [19]. Hev b 5 and Hev b 6 are recognized by IgE in a majority of both HCW and NRL-allergic children [20–23]. Two of the major NRL allergens, Hev b 2 and Hev b 13, are glycosylated proteins. For both proteins, it has been demonstrated that IgE epitopes arise on the carbohydrate moiety [24, 25]. Even though their clinical role in provoking symptoms is not widely accepted [24], both proteins elicited reactions in skin prick tests (SPTs) in a recent study [19].

In this study, we aimed to assess NRL sensitization in a group of patients with suspected respiratory allergy. An NRL extract was included within a standard panel of inhalant allergens for patients who underwent a SPT at the time of the consultation. Consistency of the results was evaluated using in vitro testing and history. Additionally, in vivo results were compared with several in vitro resources including distinct NRL allergens.

## Materials and Methods

### *Allergenic Extracts and Allergenic Molecules*

All patients underwent a standardized SPT with a commercial NRL extract (Stallergènes, Antony, France), along with a standard panel of pollen and non-pollen allergen extracts (Allergopharma, Reinbeck, Germany; Stallergènes), as previously reported [26, 27].

NRL extract and NRL extract spiked with rHev b 5 [28] were used for IgE detection on a CAP System (Phadia, Uppsala, Sweden). Since extracts were commercially available at different times during the course of the study, it was not possible to avoid different batches. *Escherichia coli*-produced recombinant molecules Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.02, Hev b 8, Hev b 9, Hev b 11, and native bromelain (nBRO) were obtained from Phadia and were used for IgE detection on the Phadia CAP System.

### *Patients, Skin Testing and Sera*

A total of 6,126 subjects, aged 2–81 years, with a male/female ratio of 1.03, were enrolled in the study. All of them were unselected consecutive subjects referred to an outpatient allergy center of the Italian National Health Service for respiratory symptoms suggesting an allergic disease. Subjects reporting symptoms related to NRL exposure were excluded from the survey. Demographical (age and gender) and clinical (respiratory and food ingestion-related symptoms) data were recorded for all patients at history taking. Patients with a positive SPT to NRL were surveyed again for experiencing any allergy symptoms when exposed to NRL-containing products. For these asymptomatic NRL-sensitized subjects, a follow-up for checking any outbreaking symptoms was established for a period between 3 and 6 years.

Patients underwent SPTs with a standardized procedure for allergenic extracts and molecules [26]. A positive reaction was recorded for a wheal of at least 3 mm in diameter and greater than the negative control measured 15 min after performing the test. SPT reactivity was classified by comparative evaluation with histamine reactivity [26]. Oral informed consent for skin testing and blood sampling was obtained from patients or caregivers during the allergy consult.

Following SPTs, sera were collected from 475 patients, either NRL SPT-positive or control patients who had granted informed consent to blood drawing for an in vitro diagnostic procedure, and stored at  $-20^{\circ}\text{C}$ .

### *IgE Detection*

Specific IgE to NRL extracts, NRL extracts plus rHev b 5 (latex+), to a panel of NRL recombinant allergenic molecules (rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9, and rHev b 11), and to native nBRO was detected by the ImmunoCAP System following the manufacturer's instructions (Phadia). nBRO, a rarely allergenic glycoprotein, was used to determine the levels of IgE to crossreactive carbohydrate determinants (CCDs) [27]. Values equal to or greater than  $0.35\text{ k}_\text{A}\text{U/l}$  were considered positive for ImmunoCAP determinations.

### *SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting*

Fresh *Hevea* latex was collected in chilled containers from rubber trees (*Hevea brasiliensis*, clone RRIM 600). Latex was centrifuged at  $44,000\text{ g}$  at  $4^{\circ}\text{C}$  for 1 h to obtain three main fractions: a top fraction containing rubber particles, an aqueous phase, the

C serum, and a bottom fraction, the B serum, containing specialized organelles called 'lutoids'. The aqueous phase was collected and centrifugation repeated. The clear aqueous C serum was freeze dried and stored at  $-20^{\circ}\text{C}$ . The bottom fraction containing the lutoids was resuspended in 0.4 M mannitol, resedimented and subjected to repeated alternate freezing and thawing to rupture the lutoids. The fluid content of the lutoids, the B serum, was then recovered by centrifugation [29]. A total of two fractions of the B serum (B1, B2) and four fractions of the C serum (C1–C4) were used for immunoblotting. These fractions were generated as follows. One hundred milligrams of the latex C serum was dissolved in 20 mM Tris/HCl, pH 7.5. Proteins were precipitated by adding  $(\text{NH}_4)_2\text{SO}_4$  to a 25%, then to a 50%, and finally to a 75% saturation. Precipitation was performed by stirring the samples for 30 min at  $4^{\circ}\text{C}$  with the  $(\text{NH}_4)_2\text{SO}_4$  and subsequent centrifugation at 12,000 g for 20 min at  $4^{\circ}\text{C}$ . Precipitates were dissolved in 20 mM Tris/HCl, pH 7.5, and supernatants were used for further precipitations. The resuspended precipitates obtained by addition of  $(\text{NH}_4)_2\text{SO}_4$  to 50 and 75% saturation were then desalted by a PD-10 column (Phadia) and applied to a MonoQ HR5/5 column (GE Healthcare, Little Chalfont, UK). Proteins were eluted using a linear gradient of 0–1 M NaCl in 20 mM Tris/HCl, pH 7.5. Proteins derived from the 50%  $(\text{NH}_4)_2\text{SO}_4$  precipitate eluted with 150–200 mM NaCl were pooled to fraction C1, proteins eluted with 300–500 mM NaCl to fraction C2. Fraction C3 contained proteins eluted with 300–450 mM NaCl, whereas fraction C4 contained proteins eluted with 450–550 mM NaCl; both C3 and C4 were derived from the 75%  $(\text{NH}_4)_2\text{SO}_4$  precipitate. Latex B serum (100 mg dissolved in 20 mM Tris/HCl, pH 7.5) was directly applied to anion exchange chromatography yielding two fractions. Fraction B1 contained unadsorbed proteins and fraction B2 proteins eluted with 150–250 mM NaCl. Protein extracts (50  $\mu\text{g}$  per lane) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, 12%) under reducing conditions and transferred to Protran nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) for immunodetection. Immunoblots were performed using 40 mM  $\text{Na}_2\text{HPO}_4$ , 7 mM  $\text{NaH}_2\text{PO}_4$ , 0.5% BSA, 0.05% w/v sodium azide, 0.5% w/v Tween-20, pH 7.5, for blocking, washing and antibody dilutions. Blocked membranes were incubated with patient and control sera diluted 1:5. Bound IgE was detected using  $^{125}\text{I}$ -labeled rabbit anti-human IgE (IBL, Hamburg, Germany), diluted 1:20. Bound immunoglobulins were visualized on Biomax MS film (Kodak, Rochester, N.Y., USA).

#### Data Management and Statistical Analysis

Data were stored in customized databases and subsequently retrieved by means of study-tailored query programs. Statistical analysis was carried out by means of GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, Calif., USA).

## Results

### Epidemiological and Clinical Data

A cohort of 6,126 subjects with symptoms suggesting an allergic respiratory disease was examined for allergy sensitization as assessed by SPT. Among the study cohort, 3,930 (64.15%) subjects presented at least one sensitiza-

**Table 1.** Epidemiological and clinical data

|                         | Total         | Latex symptoms | No latex symptoms |
|-------------------------|---------------|----------------|-------------------|
| Subjects                | 46            | 35 (76.1)      | 11 (23.9)         |
| Age, years              |               |                |                   |
| Mean                    | 33            | 35             | 25                |
| Range                   | 4–63          | 4–63           | 5–45              |
| Gender, F/M             | 34/12         | 29/6           | 5/6               |
| Occupational exposure   | 14            | 14             | –                 |
| At-risk category        | 6             | 6              | –                 |
| SPT, $\text{mm}^2$      | $6.5 \pm 1.7$ | $7 \pm 1.6$    | $4.8 \pm 1$       |
| Latex monosensitization | 7 (15.2)      | 7 (15.2)       | –                 |
| Food symptoms           | 15            | 15             | –                 |
| Other sensitizations    |               |                |                   |
| Pollens                 | 31 (67)       | 21 (60)        | 10 (91)           |
| Mites                   | 23 (50)       | 15 (43)        | 8 (73)            |
| Moulds                  | 11 (24)       | 8 (23)         | 3 (27)            |
| Mammals                 | 10 (22)       | 7 (20)         | 3 (27)            |
| Food                    | 6 (13)        | 5 (14)         | 1 (9)             |

Figures in parentheses are percentages.

tion and 46 subjects had a positive SPT with NRL extract, accounting for a 1.2% prevalence of NRL sensitization within the sensitized population and a 0.75% prevalence within the general cohort sample.

Seven out of the 46 NRL-sensitized subjects (15.2%) were sensitized to NRL alone, whereas the remaining 38 (82.6%) subjects had additional sensitizations (table 1).

When surveyed again for occurrence of respiratory symptoms upon exposure to NRL-containing products, 11 out of the 46 NRL SPT-positive subjects (23.9%) did not report symptoms nor develop any in a follow-up ranging from 3 to 6 years.

On the other hand, 35 out of the 46 (76.1%) NRL-sensitized subjects reported one or more episodes of respiratory symptoms arising upon exposure to NRL-containing products. All of them reported rhinitis, whereas 15 (32.6%) had asthma. Contact urticaria was associated with either rhinitis alone or rhinitis and asthma in 26 cases (56.5%), and no severe systemic reactions were reported. None of these patients had ever identified or suspected NRL as an eliciting factor for their symptoms when enrolled in the study.

As for occupational history, 6 out of 35 SPT-positive symptomatic subjects were HCW and could therefore be referred to as category at risk. Mild rhinitis was present in 3 cases following exposure in the workplace, whereas mild to severe asthma with associated rhinitis was pres-

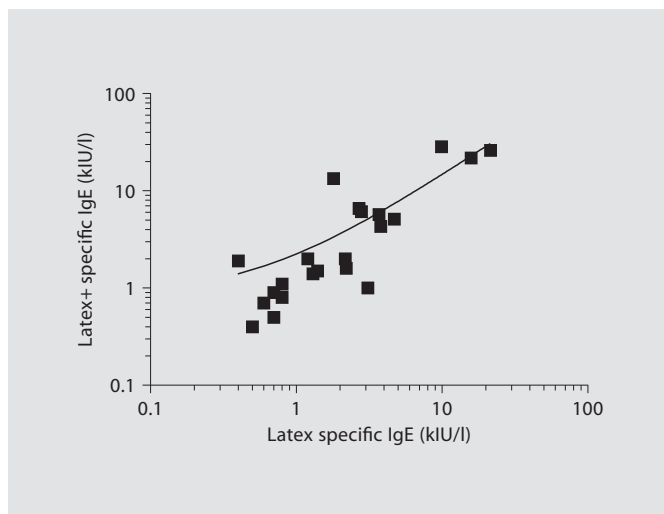


**Fig. 1.** Correlation between measurements of specific IgE with regular (latex) or Hev b 5-spiked (latex+) NRL extracts, as per linear regression analysis. A significant correlation is observed for sera positive on both determinations (total 22 sera), with  $p < 0.0001$  and  $r^2 = 0.76$ .

ent in the other 3. Episodes of mild contact urticaria were also present in 3 cases upon wearing NRL gloves for more than 10 min. Although aware of the onset in the workplace, these subjects had never suspected NRL as the eliciting factor for their respiratory symptoms.

Occupational non-HCW-related NRL exposure could be identified for 8 (22.9%) symptomatic patients (1 bartender, 1 catering staff, 2 cleaning staff, 1 massage therapist, 1 hairdresser, 1 florist, 1 lab technician), 3 of whom were exposed to fake flowers made by NRL that occurred both at home and in the workplace. In these cases, NRL-related symptoms dramatically improved after NRL source removal and/or avoidance.

A nonoccupational NRL exposure was identified for the remaining 21 out of the 35 symptomatic subjects (60%).

#### *Latex-Specific IgE Detection with NRL Extracts and Recombinant Molecules*

Twenty-nine consecutive sera were tested by means of ImmunoCAP for specific IgE towards regular NRL extract and NRL extract spiked with Hev b 5 [28]. Twenty-two subjects (76%) showed positive results, whereas no specific IgE was detected for 7 subjects (24%). Overall, results obtained with the two types of extract were very consistent with a statistically significant correlation for a  $p$  value  $< 0.0001$  (fig. 1).

Specific IgE to individual recombinant molecules was subsequently tested in 21 sera previously positive to NRL extracts. Specific IgE for at least one of the molecules could be detected in 17 (81%) cases (table 2), whereas 4 (19%) sera were totally negative to all of the molecules tested, even if positive with extracts.

Among the tested molecules, no patient displayed IgE to Hev b 1, Hev b 2 and Hev b 8, while the majority of sera reacted with Hev b 6.01 and Hev v 6.02, with positive results for 15 (77%) subjects each. Recognition of Hev b 5 was also quite frequent, having found positive results for 8 out of 17 (47%) patients (fig. 2a).

Hev v 5, Hev v 6.01 and Hev v 6.02 were not only recognized by the highest number of subjects, the highest specific IgE concentrations were also detected for these allergens (fig. 2b). Notably, in 3 sera (patients No. 3, 8 and 12) (table 2), Hev b 5 specific IgE could not be detected, despite the measurement of a higher specific IgE level for NRL extract spiked with Hev b 5. nBRO-specific IgE was detected in 5 sera, whereas only 1 of these (patient No. 17) was positive to both extracts but negative to all tested recombinant molecules.

#### *Immunoblotting*

Three sera from NRL-sensitized patients (2 positive and 1 negative for NRL-specific IgE), along with 2 control sera from subjects allergic to mould, but neither allergic nor sensitized to NRL, were used for immunoblotting with fractions of B and C serum from NRL (fig. 3). In the B2 fraction, there was no binding at all, whereas in the B1 fraction, a couple of discrete bands could be seen in the range of 30 kDa, the stronger one particularly evident for sera No. 8, which is actually the one with the highest NRL-specific IgE measurement (table 2). The overall pattern observed on the immunoblotting with fractions of the aqueous C serum fractions was very heterogeneous, and bands could also be observed for control sera, although remaining negative with internal controls.

#### **Discussion**

In this study, we tested the ability of an NRL SPT to identify missed cases of NRL allergy with the aim to evaluate the prevalence of NRL sensitization in a cohort of not-at-risk subjects. NRL extracts were included in a standard panel of inhalant allergens used for an SPT on patients referred to the allergy clinic for respiratory symptoms, excluding those who were claiming to suspect a causative role for NRL already. The observed prevalence

**Table 2.** Latex extract, latex extract spiked with rHev b 5 (latex+), and latex recombinant molecule (Hev b 1–11) specific IgE measurements (kU/l)

| Patient | Latex | Latex+ | Hev b recombinant molecules |   |     |     |      |      |   |     |     | nBRO |
|---------|-------|--------|-----------------------------|---|-----|-----|------|------|---|-----|-----|------|
|         |       |        | 1                           | 2 | 3   | 5   | 6.01 | 6.02 | 8 | 9   | 11  |      |
| 1       | 2.2   | 1.6    | –                           | – | –   | –   | 0.4  | –    | – | –   | –   | –    |
| 2       | 2.7   | 6.6    | –                           | – | –   | 2.8 | 1.2  | 0.7  | – | –   | –   | –    |
| 3       | 3.8   | 4.3    | –                           | – | –   | –   | 1    | 0.9  | – | –   | –   | –    |
| 4       | 1.3   | 1.4    | –                           | – | –   | –   | 0.7  | 0.5  | – | –   | –   | –    |
| 5       | 0.5   | 0.4    | –                           | – | –   | –   | –    | –    | – | –   | –   | –    |
| 6       | 3.1   | 1      | –                           | – | –   | –   | 0.6  | 0.4  | – | –   | –   | 3.2  |
| 7       | 0.4   | 1.9    | –                           | – | –   | 2.8 | –    | –    | – | –   | –   | –    |
| 8       | 9.9   | 28.4   | –                           | – | 0.5 | –   | 11.5 | 9.3  | – | 0.4 | 0.5 | 0.9  |
| 9       | 2.8   | 6.1    | –                           | – | –   | 4.4 | 0.9  | 1.1  | – | –   | –   | –    |
| 10      | 1.2   | 2      | –                           | – | –   | 1.9 | 0.7  | 0.4  | – | –   | 0.6 | –    |
| 11      | 21.5  | 26.1   | –                           | – | –   | 2.1 | 5.4  | 16.2 | – | 0.6 | 1.1 | 0.8  |
| 12      | 3.7   | 5.7    | –                           | – | –   | –   | 1.5  | 3    | – | –   | 0.8 | 2.1  |
| 13      | 1.8   | 13.4   | –                           | – | –   | 8.7 | –    | 1.1  | – | –   | –   | –    |
| 14      | 0.7   | 0.5    | –                           | – | –   | –   | –    | –    | – | –   | –   | –    |
| 15      | 0.8   | 0.8    | –                           | – | –   | –   | 0.5  | 0.4  | – | –   | –   | –    |
| 16      | 0.8   | 1.1    | –                           | – | –   | –   | 1    | 0.9  | – | –   | –   | –    |
| 17      | 0.7   | 0.9    | –                           | – | –   | –   | –    | –    | – | –   | –   | 0.7  |
| 18      | 15.8  | 21.8   | –                           | – | –   | 4.9 | 6.1  | 2.4  | – | –   | –   | –    |
| 19      | 2.17  | 2      | –                           | – | –   | –   | –    | –    | – | –   | –   | –    |
| 20      | 4.7   | 5.1    | –                           | – | –   | 1.3 | 2.1  | 1.2  | – | –   | –   | –    |
| 21      | 1.4   | 1.5    | –                           | – | –   | –   | 1.8  | 1.1  | – | –   | –   | –    |

CCD IgE was detected by means of nBRO.

of NRL sensitization falls a little below the range reported by previous epidemiological studies on similar study cohorts (4–6%) [7]. Females appeared to be more frequently affected than males. This could possibly be due to the fact that women are more frequently involved in occupational or home activities involving the use of NRL gloves as important means of sensitization. Further studies are needed to address housewives as a category at risk for NRL sensitization.

Taking a more detailed history, NRL could be identified as the eliciting factor for respiratory symptoms in three fourths of the cases (table 1), even though about one fourth of these NRL-sensitized patients was actually referring to be asymptomatic upon NRL exposure, as described for other allergens in other studies [30]. Furthermore, in 57% of these cases, respiratory symptoms were accompanied by urticaria, whereas for the rest of the patients – none with a history of surgical procedures – only respiratory symptoms were present. This seems to reflect different routes of sensitization and subsequent exposure [31].

As for cosensitizations encountered in the NRL-sensitive patient group, NRL-symptomatic patients tended to have an overall higher rate of sensitizations to mites, moulds and food, while asymptomatic subjects tended to have a higher rate of sensitizations to pollen (table 1). A history of adverse food reactions was referred to by 15/35 patients (43%), but no symptoms were reported upon ingestion of fruit typically described to be associated with the latex-fruit syndrome [32–34], probably due to the fact that many of those fruits (e.g., papaya and mango) are not typically eaten by the general Italian population.

The use of recombinant molecules for specific IgE detection has been proven to be a very effective tool for component-resolved diagnosis and is going to have a profound impact on the overall management of allergic diseases [35]. It was thus suggested to spike aqueous NRL extracts with Hev b 5 in order to increase their diagnostic power [28]. However, it has been reported that the use of Hev b 5 spiked extracts resulted only in a limited diagnostic improvement in sensitivity with essentially unchanged specificity [36], as recently confirmed in a large

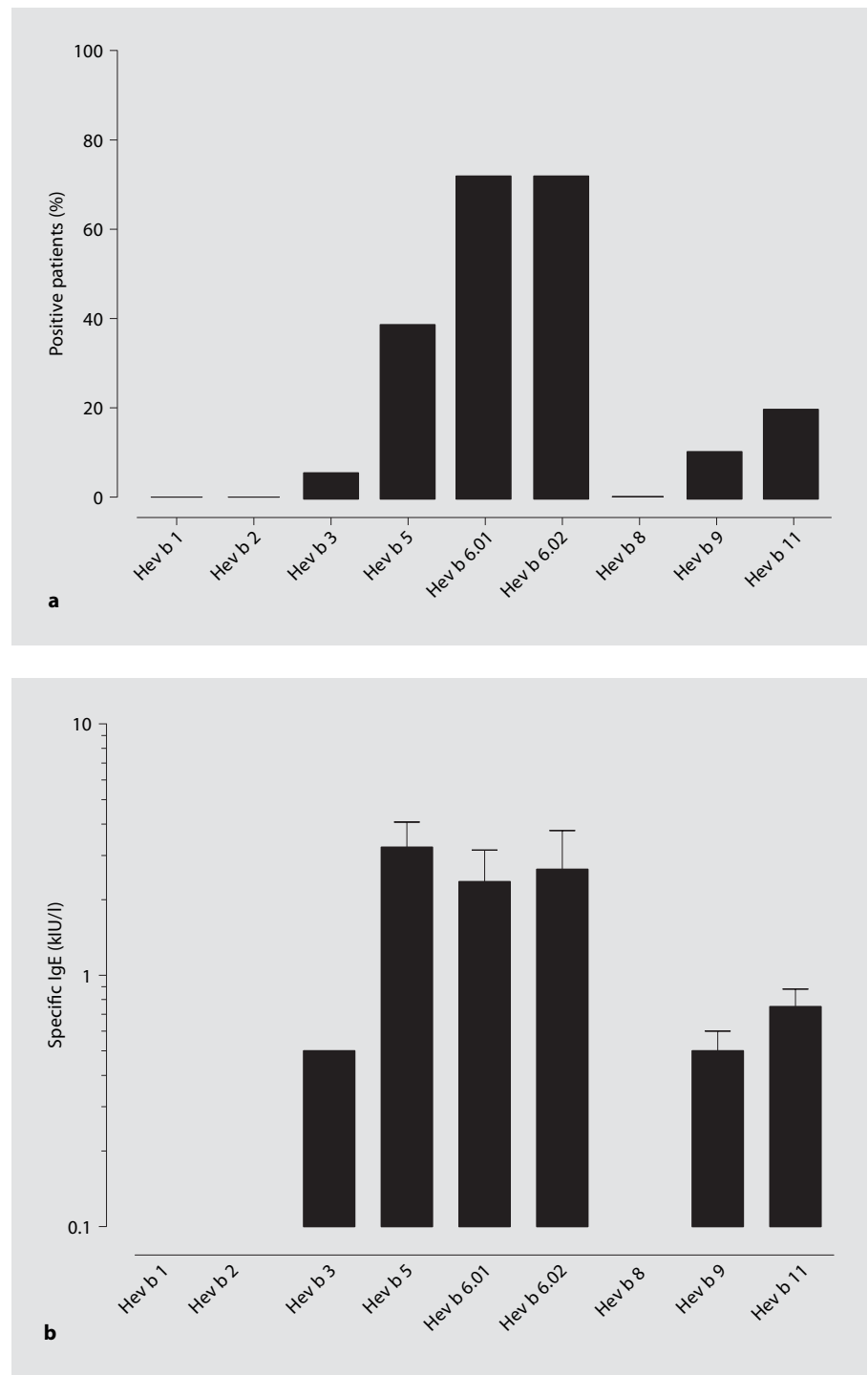
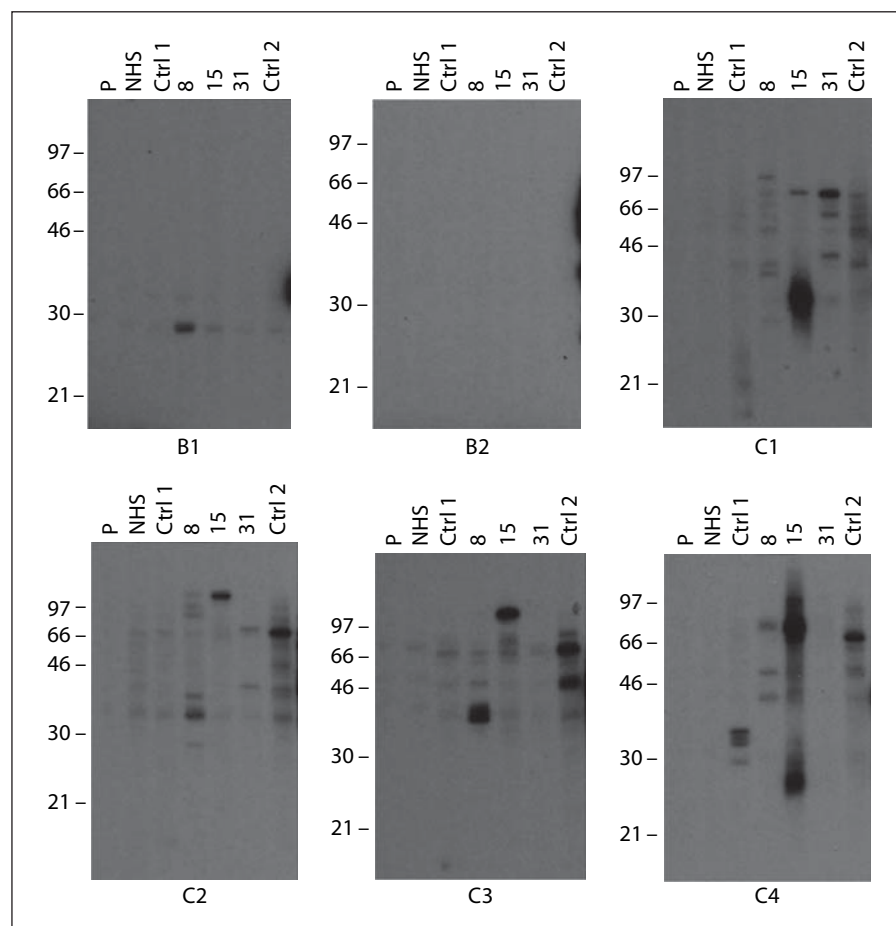


**Fig. 2. a** Prevalence of specific IgE to individual recombinant NRL molecules in patients positive for at least one molecule (total 17 patients). **b** Specific IgE concentrations to individual recombinant NRL molecules in patients positive for at least one molecule (total 17 patients). Values are expressed as means plus standard deviations considering cutoff exceeding values ( $>0.35$  kIU/l).

study on HCW [37]. Similarly, we did not find any difference in results obtained with the two different extracts, even though 3 patients (No. 3, 8 and 12) (table 2) showed higher IgE levels to the spiked NRL extract in the absence

of detectable Hev b 5 specific IgE. This finding might be explained with the use of two different batches of NRL extracts, commercially available at two different times during the study period. This could have resulted in a

**Fig. 3.** Immunoblotting with NRL B (B1, B2) and C (C1–C4) serum fractions. P = Phosphate buffer; NHS = normal healthy subjects; Ctrl 1 and Ctrl 2 = control sera from subjects allergic to mould, but neither allergic nor sensitized to NRL; 8, 15 = NRL-sensitized allergic patients (table 2); 31 = serum from a subject positive to SPT with NRL but negative on specific IgE detection both with extracts and recombinant molecules tested (not in table 2).



higher concentration of Hev b 6.01 and Hev b 6.02 in the latter used for Hev b 5 spiking, as suggested by the high measurements of specific IgE towards those two recombinant molecules.

In our study, Hev b 1, Hev b 2 and Hev b 8 appeared to have little value in the diagnosis of NRL allergy. In the case of Hev b 2, it has to be mentioned that the recombinant *E. coli* product lacks any carbohydrate structure that has been described as important IgE recognition site for this allergen [24]. Hev b 5, Hev b 6.01 and Hev b 6.02 are the most frequently recognized recombinant molecules and are able to detect specific IgE in 71% of all sensitized patients tested positive on NRL extracts (table 2). These findings partly overlap with previous reports (73.1%) [38].

The fact that on immunoblotting some bands could be identified also for negative control sera from mould-allergic patients, more evident on C fraction blots, and for control 2 serum, indicates that there is a need for further

characterization of CCDs in NRL, which might be responsible for this result [27, 39]. As for the bands that show up for sera 8, 15 and 31, they might be due either to CCD specificity (as for serum 8, testing positive for nBRO-specific IgE) or positivity towards NRL molecules not yet characterized.

Overall, our results indicate that NRL extracts should be included in standard allergen panels used for routine evaluation of patients with respiratory symptoms in order to minimize misdiagnosis or underdiagnosis of NRL sensitizations and NRL allergy, which can actually be independent of typical category at-risk condition or occupational exposure. Furthermore, our data on NRL-specific IgE and immunoblotting with NRL extract fractions show that there is a compelling need for a better characterization of extracts to better clarify the role of CCDs. Even though IgE immunoblotting gives some information on reactivity of individual sera towards NRL components, it does not provide per se any direct identification of in-

volved NRL components. More recombinant molecules need to be isolated and made available for diagnostic use, in order to further differentiate positivity patterns of specific IgE for component-resolved diagnosis. New NRL recombinant molecules would ultimately allow detection of specific IgE also in those 30% of the sera for which no detection could be attained with the use of the recombinant molecules currently available. Still, it must be considered that the use of nonstandardized NRL extracts could result in underdiagnosis of NRL patients, as recently reported

by Yeang et al. [40]. A comprehensive panel of allergens including NRL molecules on proteomic microarray platform [41] will be of great help in identifying less prevalent but still risky sensitizations in allergic patients.

## Acknowledgment

This study was supported in part by the 'Austrian Programme for Advanced Research and Technology' (APART) of the Austrian Academy of Science (to S.W.).

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